Received: 5 September 2017 | Accepted: 8 December 2017

DOI: 10.1111/1365-2435.13038

RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

Routine habitat switching alters the likelihood and persistence of infection with a pathogenic parasite

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Funding information
Natural Environment Research Council, Grant/Award Number: NE/N009800/1 and NE/N009967/1; Cambridge Overseas Trust; Risk Assessment of Chytridiomycosis to European Amphibian Biodiversity

Handling Editor: Dana Hawley

Abstract

1. Animals switch habitats on a regular basis, and when habitats vary in suitability for parasitism, routine habitat switching alters the frequency of parasite exposure and may affect post-infection parasite proliferation. However, the effects of routine habitat switching on infection dynamics are not well understood.
2. We performed infection experiments, behavioural observations and field surveillance to evaluate how routine habitat switching by adult alpine newts (*Ichthyosaura alpestris*) influences infection dynamics of the pathogenic parasite, *Batrachochytrium dendrobatidis* (*Bd*).
3. We show that when newts are exposed to equal total doses of *Bd* in aquatic habitats, differences in exposure frequency and post-exposure habitat alter infection trajectories: newts developed more infections that persisted longer when doses were broken into multiple, reduced-intensity exposures. Intensity and persistence of infections were reduced among newts that were switched to terrestrial habitats following exposure.
4. When presented with a choice of habitats, newts did not avoid exposure to *Bd*, but heavily infected newts were more prone to reduce time spent in water.
5. Accounting for routine switching between aquatic and terrestrial habitat in the experiments generated distributions of infection loads that were consistent with those in two populations of wild newts.
6. Together, these findings emphasize that differential habitat use and behaviours associated with daily movement can be important ecological determinants of infection risk and severity.

KEYWORDS

Batrachochytrium dendrobatidis, behaviour, disease risk, environmental heterogeneity, habitat use, host behaviour, host–parasite interactions

1 | INTRODUCTION

All organisms are susceptible to parasites, yet parasites do not infect all susceptible hosts equally (Wilson et al., 2002). While host susceptibility is always at least to some degree an intrinsic trait, extrinsic factors can also strongly influence the probability and strength of infection. Environmental variation among habitats that hosts move between has the potential to be an important driver of infection dynamics

because different environments associated with different habitats carry different risks of parasitism (Parratt, Numminen, & Laine, 2016). Large-scale, inter-seasonal habitat switching (i.e. migration) that exposes potential hosts to divergent environments is already known to cause spatial and temporal variation in infection (Altizer, Bartel, & Han, 2011). However, animals also switch habitats to complete essential daily activities such as foraging, mate searching and predator avoidance. The influence of this "routine" (Van Dyck & Baguette, 2005) habitat switching on infection dynamics has received much less attention. Despite the shorter time-scales involved, evidence is accumulating that these more rapid, local habitat shifts can significantly affect rates of parasitism (Byers, Malek, Quevillon, Altman, & Keogh, 2015; Hoch, Monnet, & Agoulon, 2010).

Environmental heterogeneity should act on the ability of a parasite to survive, grow and reproduce and can be broken down based on fundamental theory of transmission dynamics. Specifically, exposure frequency, parasite density and post-infection parasite proliferation should vary according to habitat suitability, and are all well-accepted drivers of infection dynamics (Anderson & May, 1991; Wilson et al., 2002). Empirical studies of the interactions among these factors are few and far between though, and it is unlikely that they would be deterministic. For example, we are unaware of any study where the frequency of exposure to infectious particles was varied while the number of infectious particles was held constant, although exposure frequency is considered to be an important driver of infection (Leon & Hawley, 2017) and disease (Rohani, Keeling, & Grenfell, 2002) dynamics. Nevertheless, each step of the host movement process should have specific impacts on both the probability and subsequent strength of infection. First, the time that a host spends in habitats harbouring parasites roughly corresponds to the number of exposure events over time (exposure frequency). Second, habitats with heavier parasite concentrations should pose a greater risk of infection than habitats where concentrations are light (exposure intensity). Third, even when parasites are absent, if a host is already infected, then occupying habitats that positively influence parasite growth and reproduction should also positively affect post-infection dynamics.

Animals choose whether to move between habitats, a decision-making process that can be influenced by the risk of parasitism. Such decisions can affect the frequency with which animals spend time in habitats that facilitate infection and post-infection parasite proliferation. Parasite avoidance behaviours are documented in a wide range of host taxa (Hoverman & Searle, 2016; Moore, 2002). Hosts may alter habitat use in response to parasites at multiple phases of the interaction, depending on the risks posed by exposure and infection, and the effect of such changes on infection dynamics likely depends on when during the interaction habitat changes are made (Byers et al., 2015; Wilson et al., 2002). Hosts may avoid parasites prior to exposure: since risk of infection often varies across habitats, avoidance may simply be a matter of preference for habitats that are less likely to carry parasites. Avoidance behaviours can also be a direct response to exposure, particularly if hosts do not easily detect parasites or habitats that inhibit parasite survival and growth are easily accessed. If the probability of exhibiting avoidance covaries to some degree with risk of infection and disease, and the effects of pathogen-inhibiting habitats are strong, hosts may switch after infections occur when parasite burdens have increased to potentially costly levels. As a result, avoidance can reduce infection risk and alter infection dynamics driven by extrinsic processes like dose strength and frequency. However, the efficacy of pre- and post-infection habitat switching for minimizing infection risk is uncertain, and under some conditions habitat switching may actually exacerbate infections (Hoodless, Kurtenbach, Nuttall, & Randolph, 2002; Morgan, Medley, Torgerson, Shaikenov, & Milner-Gulland, 2007).

In this study, we assessed the role of routine habitat switching in infection dynamics of *Batrachochytrium dendrobatidis (Bd)*, a microscopic fungus that infects keratinized epidermal cells of amphibians via free-living zoospores. *Bd* is considered a major threat to global biodiversity (Fisher et al., 2012) but has highly variable distributions within and among susceptible host species (Bielby, Fisher, Clare, Rosa, & Garner, 2015). Substantial advancements have been made in modelling *Bd* dynamics within aquatic habitats (Briggs, Knapp, & Vredenburg, 2010; Wilber, Knapp, Toothman, & Briggs, 2017). However, many adult amphibians routinely move between aquatic and terrestrial habitats. *Bd* zoospores are waterborne (Piotrowski, Annis, & Longcore, 2004), have limited mobility (Piotrowski et al., 2004), and are sensitive to environmental fluctuations like drying (Raffel, Halstead, McMahon, Davis, & Rohr, 2015), which results in heterogeneous densities of zoospores across aquatic and terrestrial habitats used by amphibians (Heard et al., 2015). Field surveillance (Kriger & Hero, 2007), broad-scale modelling (Bielby, Cooper, Cunningham, Garner, & Purvis, 2008) and experimental work (Becker et al., 2014) have established a general negative association between infection risk and host life histories that are biased towards terrestrial habitats. However, laboratory experiments have found that *Bd* can proliferate in hosts (Raffel et al., 2015) and survive outside of hosts (Kirshtein, Anderson, Wood, Longcore, & Voytek, 2007; Kolby et al., 2015) in sufficiently wet terrestrial habitats. There is also evidence for cryptic but persistent infection of terrestrial hosts (Minting, 2012) and documented cases of *Bd* infecting fully terrestrial amphibians (Kolby et al., 2015). Thus, whether increased terrestrial use can regulate either the probability of infection or post-infection parasite proliferation over short time spans associated with routine habitat switching is unclear. Avoidance of *Bd*-infected habitats has been suggested (McMahon et al., 2014) but detailed evaluations of *Bd* avoidance behaviours are lacking (Raffel et al., 2015).

We used adult alpine newts (*Ichthyosaura alpestris*) as a focal host. Alpine newts breed for prolonged periods in lakes and ponds during which newts mate promiscuously and are largely aquatic. However, both sexes sustain varying degrees of terrestrial activity during breeding periods (Weddeling, Hachtel, Sander, & Tarkhnishvili, 2004), perhaps to obtain nutrient-rich food (Denoel, 2004), avoid predators (Winandy, Darnet, & Denoël, 2015), search for different aquatic habitats (Kopecky, Vojar, & Denoël, 2010) and minimize parasitism (Todd, 2007). Field surveillance has reported *Bd* infections in wild populations of alpine newts (Ohst, Gräser, Mutschmann, & Plötner, 2011; Rasmussen, Eisenberg, Alfermann, & Köhler, 2012; Wood, Griffiths, & Schley, 2009) but with no evidence of disease or mass-mortality as in

highly susceptible hosts. However, recent experimental work with this species has shown costs of continuous exposure to *Bd* that manifest as mortality at relatively low infection levels (Miaud et al., 2016). Thus, while much exposure to *Bd* in the wild appears to be non-lethal, newts can conceivably benefit by adopting behaviours that minimize exposure to *Bd*. Our overarching aims were to establish the mechanistic basis for how habitat switching alters infection dynamics and to determine if *Bd* affects habitat switching behaviours. We first surveyed *Bd* infection in populations of adult newts during a breeding season to characterize natural within-season variation in *Bd* loads. We then conducted two experiments to test whether: (1) exposure frequency or exposure intensity had greater impact on the course of *Bd* infections; (2) habitat type (aquatic vs. water-saturated terrestrial) influenced the persistence of infections and; (3) newts behaviourally modify use of habitats in response to changes in infection risk and post-infection loads.

2 | MATERIALS AND METHODS

2.1 | Field surveys of prevalence and infection loads

We first sampled two populations of alpine newts inhabiting networks of aquatic habitats, one in the Guadarrama Mountain National Park, Spain and one in Cornwall, U.K. The Spanish network comprises permanent and ephemeral alpine ponds surrounded by moist grassland. Newts co-occur with multiple amphibian species with known histories of *Bd* infection (Bosch & Martínez-Solano, 2006). The Cornish network comprises man-made ponds in residential areas. Here, alpine newts co-occur with palmate newts (*Lissotriton helveticus*) and various anuran species, and *Bd* has been detected infecting alpine newts occupying all sampled ponds (T. W. J. Garner, unpublished data). We dipnetted ponds during the breeding season and collected *Bd* samples by rubbing sterile swabs over the venter and appendages of newts. Swabs (MWE Ltd.) were stored in 1.5 ml microtubes and transported in coolers to London for quantitative molecular detection of infection (see below).

2.2 | Experiment 1

We tested the effect of exposure frequency, exposure intensity and post-exposure habitat switching on the course of *Bd* infections in the absence of habitat choice. Male newts were collected from the Cornish sites, initially housed individually in 1.6 L plastic containers containing 750 ml of aged tap water (see Supporting Information for husbandry details). Newts had unknown infection histories but as adults inhabited a persistently risky environment for years. For this reason we used a 7-day course of antifungals (itraconazole; Garner, Garcia, Carroll, & Fisher, 2009) 1 week prior to the experiment to clear any pre-existing *Bd* infections and confirmed clearance using qPCRs before the start of experimental exposures (Boyle, Boyle, Olsen, Morgan, & Hyatt, 2004). Treatments were completed under veterinary care and all newts were deemed in good health before first exposures. Newts were fed bloodworms (chironomid larvae) twice per week during antifungal treatments and throughout the experiment. We conducted antifungal treatments and the experiment in temperature-controlled rooms (18–20°C) with regular airflow and a 16 hr daylight cycle.

We randomly assigned 90 newts to one of three exposure treatments: a negative control (3 × sham exposure to liquid media); a single high dose of 1.8×10^6 zoospores followed by two sham exposures (intense exposure treatment); or three low doses of 6.0×10^5 zoospores (frequent exposure treatment) (Supporting Information Figure S1). Therefore, newts exposed to *Bd* were exposed to the same number of zoospores, and the total volume of media was kept constant across all treatments. We exposed newts individually for four hours on Days 1, 7 and 14 in 0.07 L containers containing 35 ml of aged tap water and their respective treatment exposure and rinsed them with aged tap water afterwards before returning to their experimental housing. We exposed newts in smaller, separate containers to decrease dose dilution and eliminate the risk of environmental contamination that could influence molecular diagnostics. We used a *Bd*GPL strain (Farrer et al., 2011) isolated from an alpine newt collected in Cornwall.

During exposures, we replaced water with moistened paper towels in housing for half of the newts in each exposure treatment, which served as terrestrial replicates. We kept paper towels saturated but free of standing water by misting containers with aged tap water every other day. We changed the paper towels in terrestrial containers and changed water in aquatic containers once per week during the exposure procedures. One week after the final exposure (Day 21), we placed all terrestrial newts back into aquatic containers while keeping aquatic newts in the same containers, where they were held until the end of the experiment (Day 28). We simultaneously exposed ten captive bred and infection-free Mallorcan midwife toad tadpoles (*Alytes muletensis*), a host that is highly susceptible to infection (Doddington et al., 2013), to *Bd* according to the frequent exposure treatment to serve as a positive control for infectivity of the *Bd* culture. To assess infection, we collected epidermal swab samples (or for *A. muletensis* tadpoles, buccal swabs) on Days 1, 7 and 14 (immediately prior to exposures), 21 and 28. If the skin of terrestrial newts was dry, we dipped swabs in sterile water prior to swabbing.

2.3 | Experiment 2

We then tested the behavioural responses of newts when the total concentration of zoospores (i.e. risk of infection) was not held constant as it was in the first experiment. We used the same collection, pre-experimental antifungal treatment, *Bd* isolate and initial husbandry methods as in Experiment 1 (see Supporting Information methods).

Newts were housed individually in 5 L plastic containers divided equally into terrestrial and aquatic habitats. (Figure S2, Video S1). For terrestrial habitat we used moistened terrarium moss (Zoo Med Laboratories, Inc., CA, USA) overlaid on a pebble substrate kept saturated for the duration, and filled the aquatic habitat with 1 L of aged tap water. Pilot tests of newt activity showed that newts moved freely between habitats (data not shown).

We ran the experiment in three sequential batches of 30 newts ($N = 90$). We randomly assigned newts to one of 3 treatments (negative control, low risk, high risk), with 10 newts per treatment in each batch. Newts were given 1 day to acclimate to the tanks before experiments began. During the initial exposure, we confined newts to the aquatic portion to ensure that all newts would unavoidably experience exposure to *Bd* on the first day. We pipetted sterile liquid media (no risk control), 3.0×10^5 active *Bd* zoospores (low risk) or 3.0×10^6 active *Bd* zoospores (high risk) into aquatic habitats, removed barriers to terrestrial habitat and began video recording newt activity immediately after barriers were removed. We repeated exposures daily for 7 days after removing dirt particles or excrement from aquatic habitat.

We digitally recorded the terrestrial and aquatic activity of exposed newts with an overhead array of six webcams (Logitech C310, Newark, CA, USA), each covering the aquatic portion of 5 containers (i.e. "camera blocks") and connected to a computer (Dell Inspiron 350). Container locations were randomized across the array. We recorded time spent in the aquatic habitat (visualizing newts against the pale aquatic background was straightforward), and assumed newts spent the remaining time in terrestrial habitat. Webcams captured one image per minute during simulated daylight hours (6:00–20:00 hrs) for 8 days using iSpy webcam software (www.ispyconnect.com). Newts were then transferred to clean 1.6L containers containing 750 ml *Bd*-free aged tap water for 24 hrs to control for environmental contamination with *Bd*. Newts were then swab sampled for qPCR diagnostics.

2.4 | Parasite detection

We followed identical procedures and used the same equipment to process all samples collected for this study. We quantified the amount of *Bd* DNA on each swab in duplicate using qPCR diagnostics, appropriate negative controls (Boyle et al., 2004) and four concentration standards serving as positive controls (Bielby et al., 2015; Garner, Walker, et al., 2009; Luquet et al., 2012) (See Supporting Information methods for further details on qPCR assays). A sample was considered positive when both duplicates amplified, or when rerunning single amplifications generated a clear positive. *Bd* loads are reported here in genomic equivalents (GE), where one GE is equivalent to a single zoospore. Since newts consistently exhibited low-level infections (see Results), we considered GE values of at least 0.01 GE to be positive for infection.

2.5 | Data analysis

For Experiment 1 we used infection status (uninfected vs. infected) and infection intensity (log-transformed GE) as response variables. We first averaged individual newt values across weeks to categorize infection status and calculate mean GE and maximum GE. Here a newt was "infected" if infection was detected on Days 7, 14, and/or 21. We used generalized linear models (GLMs) to test the effect of exposure, habitat and the interaction of these two factors, using a binomial error structure when infection status was the response and a Gaussian error structure when mean and maximum *Bd* load (log-transformed) were the response. For weekly analyses we ran generalized linear mixed models (GLMMs) with weekly infection status and GE values as response variables, identical error structures and newt identity as a random effect to account for repeated measures. Three aquatic newts from the control treatment, one aquatic newt from the intense exposure treatment and two aquatic newts from the frequent exposure treatment died during the experiment. These animals did not exhibit symptoms of chytridiomycosis and were excluded from the analysis.

For Experiment 2, we based experiment day on 24-hr increments from the start time of the experiment and omitted images captured during daily cleaning and exposure times. We also omitted images during periods when webcam alignment did not afford a clear view of the aquatic habitat (see Supporting Information methods for times). We then calculated the time to first departure to terrestrial habitat (t_{depart}) and the proportion of time spent on land ($t_{terrestrial}$). For t_{depart} we identified the first image in which individuals were absent from the aquatic habitat. We then divided the position of this photograph along the sequence by the total number of images. Thus, individuals that never left the aquatic habitat had a value of 1, and t_{depart} decreased with faster departure times. This proportion corrected for variation in total duration of the experiments between batches that arose from differences in cleaning times. We then estimated the proportion of total images in which individuals were present in the aquatic portion of the tank ($t_{aquatic}$). We calculated $t_{terrestrial}$ as: $1–t_{aquatic}$.

To ascertain if infection risk did vary on the basis of dose strength, we fitted separate GLMs with exposure treatment as a fixed effect: one with a binomial error structure and infection status on Day 9 as the response variable, and another with a Gaussian error structure and infection intensity exhibited on Day 9 as the response variable. We omitted newts in the control treatment from these models, as these individuals were not exposed to *Bd* at any time during the experiment.

To assess the effects of risk and infection on $t_{terrestrial}$ and t_{depart} we fitted a GLM with a Gaussian error structure with cumulative $t_{terrestrial}$ (square root arcsine transformed) and t_{depart} as variables, respectively, with exposure treatment, infection status on Day 9 (0 = uninfected, 1 = infected) and GE on Day 9 as fixed effects.

We also assessed the effects of risk and infection on daily $t_{terrestrial}$ by fitting GLMMs with Gaussian error structures, $t_{terrestrial}$ (arcsine transformed) as the response variable and newt identity as a random effect to account for repeated measures of individuals. We included experiment day and its interaction with each factor (risk level, infection status on Day 9, infection intensity on Day 9) in GLMMs to consider temporal variation in effects of exposure and infection. Our *Bd* culture completed a full growth cycle in 4 days (Daversa pers. obs.) so to consider phase-specific effects on cumulative and daily $t_{terrestrial}$ we also fitted separate GLMs (for overall activity) and GLMMs (for daily activity) for two phases: Days 1–3 and Days 4–7. We included camera block as a categorical fixed effect (there were too few levels to model it as a random effect) in all GLMs and GLMMs used for the Experiment 2 analysis to account for potential spatial effects.

In all statistical analyses GEs were normalized with a $\log_{10}$ transformation, and analyses for infection intensity as the response omitted uninfected newts. We included camera block as a categorical fixed

effect (there were too few levels to model it as a random effect) in all GLMs and GLMMs. Effects of body size and weight of newts were not considered, as these variables did not differ among exposure or habitat treatments in either experiment (see Supporting Information results). For both experiments we tested our hypotheses by comparing models including factors of interest with models omitting these factors, using likelihood ratio tests for GLMs (χ^2 for GLMs with binomial error structures and *F* for GLMs with Gaussian error structures) and Kenward–Roger approximations for GLMMs. We performed all analyses in R version 3.0.1 and used the LME4 package to run GLMMs. We used the *dropterm* function in the MASS package for model comparisons and the PBKRTEST package for Kenward–Roger approximations. The results for all statistical analyses report the *M* ± *SE*, unless otherwise noted.

3 | RESULTS

3.1 | Field surveys

Wild newts consistently exhibited low-level infections [Spanish population (*N* = 49): range 0.02–24.46 GE, *M* ± *SE* = 3.53 ± 0.87 GE; UK population (*N* = 23): range 0.04–56.94 GE, *M* ± *SE* = 5.45 ± 2.57 GE; Figure S3].

3.2 | Experiment 1

All newts tested negative for *Bd* when experiments began. Nine out of ten of the *A. muletensis* tadpoles developed infections averaging 145.07 ± 128.67 GE, confirming the infectivity of our *Bd* culture. An aquatic newt in the frequent exposure treatment exhibited an outlier *Bd* load (127.3 GE) on Day 21. Removing this newt from the analysis did not qualitatively affect the results (see Supporting Information results).

Bd loads exhibited by newts in Experiment 1 were within the range of *Bd* loads in wild populations (Figure S3). Newts repeatedly exposed to low doses of *Bd* were more likely to develop infections than newts exposed to a single, intense dose (dropping exposure treatment from the GLM reduced goodness-of-fit: $\chi^2_1 = 5.87$; $p = .015$; Figure 1a), though mean *Bd* loads (intense GE = 0.67 ± 0.31; frequent GE = 4.03 ± 3.24; GLM, $F_{1,16} = 0.11$; $p = .749$) and maximum *Bd* loads (intense GE = 1.53 ± 0.59; frequent GE = 10.46 ± 9.00; GLM, $F_{1,16} = 0.01$; $p = .957$) did not differ among exposure treatments. Only frequently exposed newts exhibited infections by the end of the experiment (Figure S4 a,b). There was a significant interaction between week and exposure treatment, as the likelihood of infection of frequently exposed newts increased in later weeks (see Supporting Information results). Neither weekly mean nor maximum *Bd* loads of infected newts differed between exposure treatments (Figure S4).

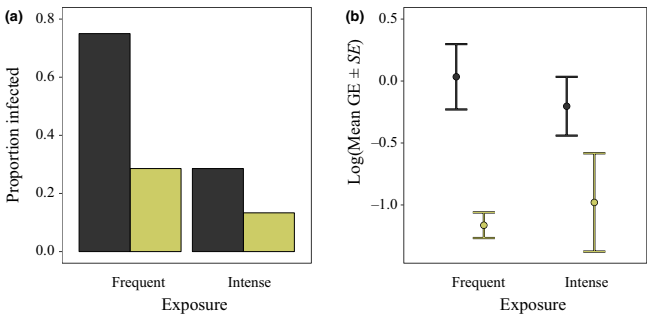

FIGURE 1 The overall proportion of infected newts (a) and *M* ± *SE Bd* load (b) among aquatic (black boxes) and terrestrial (green boxes) newts after either a frequent exposure or intense exposure in Experiment 1. Frequent exposure consisted of three low-concentration exposure events (Days 1, 7, 14), and intense exposure consisted of a single exposure (Day 1) that was three times the concentration administered to frequently exposed newts. Total exposure dose was therefore equal across exposure treatments

Post-exposure habitat also affected overall infection prevalence ($\chi^2_1 = 6.77$; $p = .009$, Figure 1a). Terrestrial newts developed weaker infections, both in terms of average *Bd* loads (aquatic GE = 4.30 ± 3.22; terrestrial GE = 0.10 ± 0.03; GLM, $F_{1,16} = 11.76$; $p = .003$; Figure 1b) and maximum *Bd* loads (aquatic GE = 11.83 ± 9.63; terrestrial GE = 0.24 ± 0.10; $F_{1,16} = 15.91$; $p = .001$). Effects of habitat were also apparent on a weekly scale (see Supporting Information results). Terrestrial newts cleared infections more quickly than aquatic newts following intense exposures (Figure S4).

Two frequently exposed terrestrial newts that previously tested negative developed detectable but weak infections on Day 28, 1 week after being returned to aquatic containers (GE = 0.14 ± 0.01; Table S1). Four aquatic newts exposed in the same manner also exhibited infections on this day, though all of these individuals previously tested positive. None of the terrestrial or aquatic newts that were exposed to a single, intense dose of *Bd* exhibited infection on Day 28 (Table S1).

3.3 | Experiment 2

All newts tested negative for *Bd* when experiments began, and newts in the control treatment did not develop detectable infections during the experiment. *Bd* loads exhibited by newts were within the range of *Bd* loads in wild populations (Figure S3). Dose strength predicted infection risk: newts in the high dose tanks were more likely to develop infections (GLM; $\chi^2_1 = 18.44$; $p < .001$, Figure 2a) and developed stronger infections (low dose GE = 0.44 ± 0.15, high dose = 8.82 ± 2.72, GLM, $F_{1,51} = 24.67$, $p < .001$; Figure 2b).

Risk did not affect how quickly newts first switched to terrestrial habitat (no risk $t_{depart} = 0.54 \pm 0.08$, low risk $t_{depart} = 0.55 \pm 0.09$, high risk $t_{depart} = 0.70 \pm 0.08$, GLM, $F_{1,51} = 1.66$, $p = .196$). Neither risk, infection status, nor infection load significantly affected cumulative $t_{terrestrial}$ (Table S2) or when breaking analysis down by *Bd* growth phases (Table S2). Terrestrial activity of newts differed between *Bd* growth phases, however (Table S3a). Both infected and uninfected newts decreased daily proportional time in terrestrial habitat throughout phase 1 (Figure 3), with no effect of infection status or load (Table S3b, Figure 3). In contrast, throughout phase 2 infected newts spent more time out of the water than uninfected newts (Table S3c; Figure 3a), with newts exhibiting stronger infections spending the most time on the terrestrial habitat (Table S3c, Figure 3b). Interactions with day for both factors reflect the predominance of these effects at the end of the second phase (Figure 3).

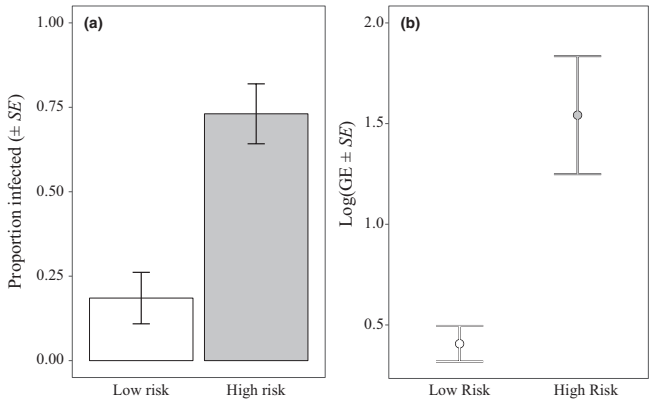

FIGURE 2 (a) Overall prevalence of *Bd* infection and (b) infection levels that infected newts exhibited on Day 9 of Experiment 2 following exposure to a low concentration (white bars) or a high concentration (grey bars) release of active *Bd* zoospores into aquatic habitat on Days 1–7. Error bars denote the standard error about the mean

4 | DISCUSSION

Our first experiments demonstrated effects of exposure frequency and post-exposure habitat on the course of newt infections, and the findings indicate that discontinuous occupancy of fully aquatic habitats harbouring *Bd* reduces infection risk. While all newts were exposed to an equivalent number of zoospores, breaking the dose into multiple events produced more infections than did a single, intense exposure. Thus, infection risk for newts is not only a function of total zoospores to which newts are exposed (Experiment 2) but also how frequently newts are exposed to zoospores over time (Experiment 1). By extension, continuous and prolonged exposure would be most likely to manifest as increased mortality, and in support of this, a recent study showed how exposing newts constantly to an infected reservoir generated significant mortality (Miaud et al., 2016).

Removal from the aquatic environment not only reduced the likelihood that newts contracted infections but also infection intensity and persistence. Despite the known suitability of well-moistened terrestrial substrates to provide adequate moisture for *Bd* (Farrer et al., 2011; Garner, Walker, et al., 2009; Raffel et al., 2015), these results suggest that even saturated terrestrial habitats can be less suitable for *Bd* than aquatic habitats, perhaps depending on the type of substrate (e.g. soil vs. moss) or the overall resistance of the host species to *Bd* infection. Emergence of infections after returning terrestrial newts to aquatic habitats was rare, indicating that the majority of hosts completely cleared their *Bd* infections while in the terrestrial habitat.

While theoretical models of *Bd* dynamics have explained the occurrence of low-level *Bd* infections in host populations by assuming low rates of zoospore production (Briggs et al., 2010) and high levels of host resistance (Wilber et al., 2017), the effects demonstrated in our first experiment suggest that escape (Altizer et al., 2011) and recovery (Shaw & Binning, 2016) from infection during periods of terrestrial activity could also generate these patterns in semi-terrestrial hosts. Accounting for periods that newts spend outside of aquatic habitat, our experiments generated infection patterns that were consistent with patterns in two populations of wild newts, emphasizing the ecological relevance of our experimental infections. In light of this overlap between the distributions of field and laboratory infection loads, we propose that routine habitat switching by newts is a likely driver of *Bd* dynamics in natural populations. Future work can test this hypothesis by considering factors not tested in this study, such as prior infection history and social behaviours in aquatic vs. terrestrial habitats.

The effects of within-season habitat switching may also have implications for community-scale host–parasite dynamics. Theory predicts that the persistence of multi-host parasites like *Bd* is dictated by the contribution of all host species to parasite reproduction (Fenton, Streicker, Petchey, & Pedersen, 2015). Although newts are a dominant species at our sites, our findings indicate that their fluctuating occupancy of aquatic habitats lessens the actual contribution of this host to the maintenance of *Bd* in the host species community. Furthermore, partial or full clearances of infection during periods of terrestrial activity detract from the pool of aquatic zoospores available to infect other hosts. As such, we expect that spillover transmission from alternative fully aquatic hosts, like the midwife toad tadpoles used as a positive control in our experiments, is important for maintaining *Bd* in communities with adult alpine newts.

Although terrestrial habitats may provide a refuge for newts to escape *Bd* infection, our second experiment indicated that newts do not actively avoid becoming infected but may modulate time in aquatic habitats containing infective *Bd* zoospores once infections proliferate. These findings support growing evidence that parasites influence daily activities of hosts and sheds new light on the topic: rather than the level of infection risk or even the infection status of hosts (infected vs. uninfected), in certain conditions host decision-making in parasitized habitats may be best explained by the intensity of infections. Such latent changes in habitat use could be indicative of threshold infection levels for parasite detection by the host, or alternatively could arise from costs of avoiding parasitized habitats. For example habitats less suitable for parasites may pose heighted risk of predation (Raffel, Hoverman, Halstead, Michel, & Rohr, 2010). Additionally, for many animals, habitats posing high infection risk also provide essential resources for reproduction and foraging. In the case of newts, fully aquatic habitats are required for mating and offspring development. Since *Bd*-induced mortality appears to be a function of infection loads rather than infection status in various amphibian species (Stockwell, Clulow, & Mahony, 2010; Wilber et al., 2017), and since newts can reduce or even remove infections by switching to adjacent terrestrial habitat (as demonstrated in Experiment 1), the reproductive and energetic consequences of avoiding *Bd* exposure may be more costly than becoming infected. Given the conflicts that can arise from avoiding parasite exposure, and since most parasite infections do not deterministically lead to death, load-dependent rather than risk-dependent adjustments in routine habitat use may be an expected strategy for many wildlife species.

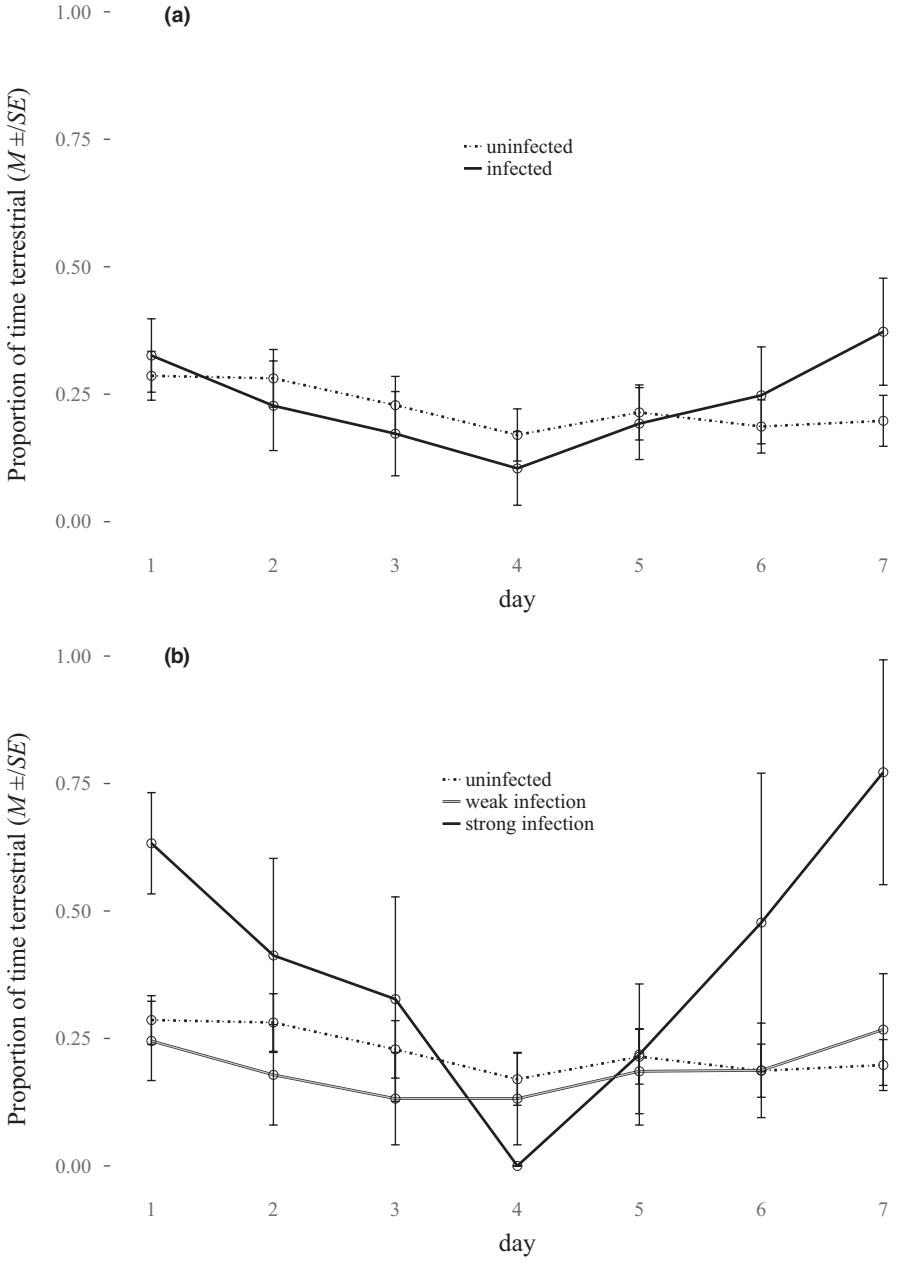

FIGURE 3 The mean proportion of recording time that newts occupied terrestrial habitat as opposed to aquatic habitat throughout the 7 days of Experiment 2, with newts distinguished by (a) infection status and (b) infection intensity exhibited on Day 9. "Weak infections" (white bars) denote those of less than 15 GE and "strong infections" (black bars) denote those of 15 GE or higher (though infection intensity was a treated as a continuous explanatory variable in data analyses). Error bars indicate the standard errors about the means (points)

5 | CONCLUSIONS

Habitats comprising natural animal populations are rarely homogeneous, and ecologists widely acknowledge that individuals vary in routine use of different habitats (Van Dyck & Baguette, 2005). Far less is known about how this potential variation in abiotic and biotic factors may affect parasitism. The results of this study suggest that hosts whose occupancy of parasitized habitats fluctuates on a routine basis face reduced risks of potentially lethal infections. Disease models that neglect short-term fluctuations in host occupancy may therefore overestimate the direct impact of parasites in host populations. Nevertheless, the observed influence of *Bd* loads on newt habitat switching emphasizes that non-lethal effects of parasites may still occur in hosts that show limited disease symptoms and in certain contexts may depend more strongly on infection proliferation than infection risk.

ACKNOWLEDGEMENTS

We thank the Cambridge Overseas Trust and the Risk Assessment of Chytridiomycosis to European Amphibian Biodiversity (RACE) for funding this study; Nicola Morris and Richard Griffiths for facilitating the collection of newts, Gonçalo Rosa and Christopher Durrant for support with animal husbandry, Giulia Vecchione for assisting with the molecular analysis, and Andy Fenton for his comments on the manuscript. D.R.D, A.M. and T.W.J.G are currently funded by NERC standard grants NE/N009800/1 and NE/N009967/1.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

D.R.D. formulated the hypothesis. D.R.D., A.M., J.B., J.W.J. and T.W.J.G. designed the experiments. D.R.D. executed the experiments. D.R.D., J.W.J. and A.M. analysed the data. D.R.D. wrote the initial manuscript, which was revised according to the comments of A.M., T.W.J.G., J.W.J. and J.B.

DATA ACCESSIBILITY

Data and codes are archived in Dryad Digital Repository https://doi.org/10.5061/dryad.d4q54 (Daversa et al., 2017).

ETHICAL STATEMENT

All experimental work and treatment with itraconazole was approved by the Zoological Society of London's Ethics Committee before commencement and licensed by the Home Office (PPL 80/2466 to Garner, PIL 70/25118 to Daversa). Field surveys at our Spanish field sites were conducted with permission from the governing department for the Environment of Comunidad de Madrid and in accordance with Park regulations. Field surveys in the United Kingdom were carried out with permission of the landowners.

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How to cite this article: Daversa DR, Manica A, Bosch J, Jolles JW, Garner TWJ. Routine habitat switching alters the likelihood and persistence of infection with a pathogenic parasite. *Funct Ecol*. 2018;32:1262–1270.
https://doi.org/10.1111/1365-2435.13038